IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Tsang et al.

Serial No.: 09/633,034 Examiner: Helms, L.R..

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For : MONOCLONAL ANTIBODIES AGAINST HUMAN COLON CARCINOMA-ASSOCIATED ANTIGENS AND USES THEREFOR

DECLARATION OF DR. JEFFREY FASICK UNDER 37 C.F.R. §1.132

I hereby certify that this paper is being deposited on June 17 2005 with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450

Lisa B. Kole
Attorney Name

35,225
PTO Registration No.

Signature

6/17/05
Date of Signature

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
MAIL STOP RCE

Sir:

I, Dr. Jeffrey Fasick, declare the following:

1. I am Director of Science of International Bioimmune Systems, Inc. ("IBS"), assignee of the above-identified application.

2. I hold a Ph.D. from the Department of Biological Sciences of the University of Maryland, and have a substantial research background in the field of molecular biology, as illustrated by my *Curriculum Vitae*, a copy of which is attached hereto.

3. This declaration relates to studies that examine the interactions between mAbs 31.1 (murine) and chimeric 31.1 (human), with a Hollinshead colon

adenocarcinoma TAA (Tumor Associated Antigen) vaccine preparation, which has been stored in our –80°C freezer at IBS. There is no log recording the date of receipt of this sample. The individual tubes and the cryobox containing them are clearly labeled as follows: Colon adenocarcinoma TAA vaccine, Lot 3, 300 ug/0.2 ml., FOR INVESTIGATIONAL USE ONLY, and are dated 2/19/86. This is presumably Hollinshead TAA vaccine as described in Hollinshead et al. (1985) in the journal Cancer 56:80.

4. The following experiments were performed at IBS within the last 2 months. The Hollinshead colon adenocarcinoma TAA vaccine was thawed at room temperature and was submitted to gradient gel SDS PAGE electrophoresis under non-denaturing conditions. Total proteins on the gel were examined by eye after staining with Coomassie Blue as well as with silver stain to determine if protein degradation had occurred during cryo-storage. The gels show two distinct and prominent major protein bands at around 60 and 70 kD with distinct minor bands ranging from <10 kD to >100 kD. Based on these gels, it is my opinion that the proteins in the Hollinshead colon adenocarcinoma TAA vaccine are intact and have not been degraded to any significant degree with time.

5. Both mAb 31.1 and mAb chimeric 31.1 bind to an antigen found in both the LS174T colon and AsPC1 pancreatic tumor cell lysates. From western blot analysis, the molecular weight of the non-denatured antigen in both tumor cell lysates has been established to be a discrete, non-diffuse band at ~40 kD. These tumor cell lysates were used as positive controls in a series of western analyses of mAb 31.1 and mAb chimeric 31.1 with the Hollinshead colon adenocarcinoma TAA vaccine. Initially, we observed that mAb chimeric 31.1 was positive for the Hollinshead colon adenocarcinoma TAA vaccine with a major band >100 kD and a minor band of around 50 kD while mAb 31.1 did not result in a positive western for the Hollinshead colon adenocarcinoma TAA vaccine at all. This struck us as odd until we hypothesized that the positive bands resulting from the mAb chimeric 31.1 western were not resulting from an interaction between mAb chimeric 31.1 and antigen but rather from the secondary mAb (goat anti-human IgG) used to develop the western and were in fact human antibodies that had

surprisingly been retained in the preparation of the Hollinshead vaccine. To examine this, we performed another western in the absence of mAb chimeric 31.1, incubating the blot only with an anti-human IgG secondary antibody. This blot resulted in the exact same banding pattern as the blot that had been incubated with mAb chimeric 31.1 as a primary antibody proving our hypothesis correct: The large molecular weight protein that we thought was the antigen target of mAb 31.1 was in fact only human IgG retained in the vaccine preparation.

6. To summarize, neither mAb 31.1 (murine) nor mAb chimeric 31.1 (including CHO31.1) react with proteins (i.e. result in positive bands as seen by Western analysis) within the Hollinshead colon adenocarcinoma TAA vaccine samples in our freezer that have been tested thusfar. There may be two reasons for this result: 1) the antigen for mAbs 31.1 and chimeric 31.1 may be degraded thereby not allowing for mAb 31.1 binding. This is highly unlikely due to the sharp banding pattern seen from the coomassie and silver stained SDS-PAGE gels of the tested vaccine preparations. To be sure, however, we are currently performing westerns with internal controls utilizing an anti-human actin mAb to confirm the integrity of proteins contained in the Hollinshead colon adenocarcinoma TAA vaccine, especially those in the 40kD molecular weight range; 2) The Hollinshead colon adenocarcinoma TAA vaccine does not contain the antigen against which mAbs 31.1 and chimeric 31.1 are directed.

7. I am familiar with United States Patent No. 5,688,657 ("the '657 patent"), and understand that it refers to Hollinshead (1985) in its method for preparing immunogen. The fact that the Hollinshead TAA vaccine in the IBS freezer does not react with monoclonal antibodies covered in the '657 patent may be explained, in my opinion, by the fact that even though the protocols used to prepare immunogen were presumably similar, they were not necessarily the same; further, I believe that the tumor sources were not the same.

8. With regard to antibody 33.28, data is currently being accumulated.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from the above-captioned patent application.

Dated: _____ By: _____
Jeffrey Fasick

Jeffry I. Fasick, Ph.D.

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Port Washington, New York 11050

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1. Employment History

2003-Present **Director of Science**
International Bioimmune Systems, Inc.

225 West Community Drive Great Neck, New York 11021
Duties: In Charge of All R&D Labs and Personnel; Responsible for Lead Product Development

2002-03 Senior Research Scientist

International Bioimmune Systems, Inc.
225 West Community Drive Great Neck, New York 11021
Research: Development of Immunotherapeutic mAbs Specific for Colon and Pancreatic Tumor Associated Antigens

1999-2002 **Scientific Consultant, Procotor and Gamble Pharmaceuticals**
G-Protein Coupled Receptor Group, Dr. Robert Barnett (Group Head)
Health Care Research Center
Proctor and Gamble Pharmaceuticals, Mason, Ohio 45040
Research: Cloning, Expresion, and Purification of the Human Melanocortin-4 Receptor Gene

1998-2002 **Postdoctoral Fellow**
Laboratory of Professor Daniel D.Oprian (Department Chair)
Department of Biochemistry and the Volen Center for Complex Systems
Brandeis University Waltham, Massachusetts 02454
Research: Molecular Mechanisms of Spectral Tuning in Mammalian Photoreceptor Visual Pigments

2. Education

1993-98 **Ph.D.**
Department of Biological Sciences
University of Maryland Baltimore County
Baltimore, Maryland 21250

1990-93 **M.S.**
Department of Biological Sciences
University of Maryland Baltimore County
Baltimore, Maryland 21250

1984-88 **B.S.**

Department of Ecology, Ethology, and Evolution
University of Illinois, Urbana-Champaign
Urbana, Illinois 61801

Experience / Accomplishments

Director of Science — **International Bioimmune Systems, Inc.**
June 2003-Present — **Great Neck, New York**

- Wrote Pre-IND briefing document for a Phase 1 clinical trial
- Represented sponsor and presented at Pre-IND meeting with the FDA
- Wrote the International Bioimmune Systems, Inc. Executive Summary Statement
- Produced the International Bioimmunce Systems, Inc. Research Summary Presentation
- Evaluated/contracted out-sourcing partners for GMP manufacturing, tissue cross-reactivity testing, and animal studies per FDA recommendations
- Presented data, research, and records to potential investors and collaborators
- Presented current research at annual shareholders meetings
- Liaison for Principle Investigators for Phase 1 Clinical Trials
- Designed/implemented S.O.P. protocols
- Designed/implemented cGMP-like manufacturing protocols for immunotherapeutic mAbs
  - High expression/high viability in stirred reactor
  - Multi-step purification with robust viral removal
  - Asceptic Fill/Finish
  - Quality Control Testing
- Initiated and supervised the immunopurification of tumor associated antigen for identification by MALDI MS
- Initiated and supervised the characterization of immunotherapeutic mAbs by tissue cross reactivity testing, cell flow cytometry, cell based ELISA assays, cellular cytotoxicity assays, animal models including pharmacokinetic and toxicity studies
- Reviewed and edited patent applications, reissues, supplements, and office actions in coordination with patent attorneys and agents
- Created alliances with contractors for all facility maintenance, repairs, and services
- Collaborations: D. Webb (Wellesley University), Oncovation (Brooklyn, NY)

Senior Research Scientist — **International Bioimmune Systems, Inc.**
June 2002-June 2003 — **Great Neck, New York**

- Developed cell line expressing immunotherapeutic mAb against colon/pancreatic cancer
  - Developed human-murine chimeric immunotherapeutic mAb
  - Developed stable expression immunotherapeutic mAb clones in CHO_{dhfr-} cells
  - Amplified immunotherapeutic mAb expression levels in CHO_{dhfr-} cells
  - Adapted mAb expressing CHO_{dhfr-} cells to suspension and serum-free media
  - Created master and working cell banks
  - Deposited cell lines and plasmids with ATCC
  - Applied for patent supplement
- Developed clonogenic assay for immunotherapeutic mAbs
- Acquired expertise in column chromatography for protein purification
  - Immunoaffinity
  - Ion Exchange
  - Gel Filtration
  - FPLC
- Worked closely with outsourcing partner on the manufacturing of immunotherapeutic mAb

- Cloned and expressed tumor associated antigen specific for immunotherapeutic mAb
- Characterized the glycosylation pattern of tumor associated antigen
- Collaborations: I. Wang (Medical University of South Carolina), K. Tsang (National Cancer Institute), J. Schubert (Cell Trends, Inc.)

Postdoctoral Fellow **Brandeis University**
April 1998-June 2002 **Waltham, Massachusetts**

- Developed large scale expression and purification system to study G-protein coupled receptors (GPCRs) from mammalian cells and suspension cultures
- Designed custom media utilized in N^{15} labelled protein exression system and expressed novel GPCRs for subsequent NMR analysis
- Performed site-directed mutagenesis to examine protein/ligand interactions and structural confirmations of GPCRs
- Performed G-protein and kinase binding assays to study protein activity
- Performed transgenic xenopus preparations for the expression of human GPCRs
- Performed DNA cloning, expression, and characterization of a variety of novel mammalian visual pigment genes
- Performed UV/visible light spectroscopy to analyze the spectral tuning properties of visual pigments
- Supervised graduate student rotation projects
- Collaborations: T. Smith (SUNY Stonybrook), M. Applebury (Harvard Medical School)

Research Fellow **University of Maryland Baltimore County**
September 1993-March 1998 **Baltimore, Maryland**

- Prepared and screened cDNA libraries
- Conducted large- and small-scale plasmid preparation in E. coli
- Utilized stable and transient transfection techniques in mammalian cell lines
- Designed and implemented protocols to secure fresh tissue from dead stranded marine mammals
- Designed technique to dissect and process fresh tissue for total RNA and genomic DNA extraction
- Acquired expertise in PCR and RT-PCR; Maxam-Gilbert and Sanger dideoxy DNA sequencing; Northern, Southern, and Western blot analysis techniques, restriction fragment analysis; autoradiography,
- Performed phylogenetic analyses using PHYLIP, CLUSTAL V, MEGA, GCG Wisconsin package, and PAUP*
- Performed DNA analysis using FASTA, BLAST, and DNA Strider
- Skilled in Powerpoint, EXCEL, Kaleidagraph, Adobe, Chemdraw, Rasmol, and WebLab Veiwer
- Acquired expertise in light and electron microscopy (SEM & TEM).
- Utilized the training of computer generated neural networks
- Acquired expertise in performing immunoaffinity chromatography
- Collaborations: National Aquarium in Baltimore, National Marine Fisheries, Chicago Zoological Assn., H. Howland (Cornell University), Virginia Marine Science Museum, Smithsonian Institute National Museum of Natural History, Maryland DNR

Additional Training

- Protein Chromatography and FPLC Seminar Series, October 11-15, 2004, GE Healthcare (Amersham Biosciences)
- Good Manufacturing Practices, February, 2004, Schiff & Company, West Caldwell, NJ

- Cell & Tissue Reactor Engineering. July 15-18, 2002. Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN.
- Cell Culture Techniques. June 25-28, 2002. The Biotechnical Institute of Maryland, Inc. University of Maryland-Baltimore. Baltimore, MD.
- Workshop on Transgenics. June 2-3, 1999. Biotechnology Center, University of Conneticut, Storrs, CT.
- Workshop on Molecular Evolution. August 3-15, 1997. Marine Biological Laboratory, Woods Hole, MA.

3. Publications

- Fasick JI, Applebury ML, Oprian DD. Spectral tuning in the mammalian short-wavelength sensitive cone pigments. *Biochemistry*. 2002 May 28;41(21):6860-5.
- Fasick, JI, Robinson PR. Cloning and expression of dolphin opsin sequences and a mechanism of sprectral tuning. Cell and Molecular Biology of Marine Mammals, Pfeiffer, C.J., Ed., Krieger Press, Melbourne, 2002.
- Fasick JI, Robinson PR. Spectral-tuning mechanisms of marine mammal rhodopsins and correlations with foraging depth. *Vis Neurosci*. 2000 Sep-Oct;17(5):781-8.
- Fasick JI, Lee N, Oprian DD. Spectral tuning in the human blue cone pigment. *Biochemistry*. 1999 Sep 7;38(36):11593-6.
- Cronin, TW, Fasick, JI, Howland, HC. Video photoretinoscopy of the eyes of the small odontocetes (*Tursiops truncates, Phocoena phocoena, and Kogia breviceps*). *Mar. Mamm. Science* 1998 14: 584-90.
- Fasick JI, Cronin TW, Hunt DM, Robinson PR. The visual pigments of the bottlenose dolphin (*Tursiops truncatus*). *Vis Neurosci*. 1998 Jul-Aug;15(4):643-51.
- Fasick JI, Robsinson PR. Mechanism of spectral tuning in the dolphin visual pigments. *Biochemistry*. 1998 Jan 13;37(2):433-8.

4. Published Abatracts

- Fasick, JI, Oprian, DD. 2001. Spectral tuning in the mammalian short-wavelength sensitive cone visual pigments. FASEB Biophysics 80: 601a.
- Fasick, JI, Robinson, PR. 1996. Molecular cloning and characterization of visual pigments in the bottlenose dolophin (*Tursiops truncates*). Society for Neuroscience 22: 792.16.

Departmental Seminars/ Invited Talks

- 10th International Conference on Retinal Proteins. August 20-24, 2002. University of Washington, Seattle, WA. Title: Spectral Tuning in the Mammalian SWS-1 Cone Pigments.
- Department of Zoology, University of New Hampshire, Durham, NH. March 1, 2002. Title: Spectral Tuning in the Mammalian Short-Wavelength Sensitive Cone Pigments.
- 25th Annual Meeting of IMATA. October 20-25, 1997. Baltimore, MD. Title: The Visual Pigments of the Bottlenose Dolphin (*Tursiops truncates*).